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Naturalization of Salt Marsh Restoration Sites in the Elizabeth River, Virginia, Assessed by Feeding Activity and Trophic Level of Mummichogs (*Fundulus Heteroclitus*)

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NATURALIZATION OF SALT MARSH RESTORATION SITES IN THE ELIZABETH RIVER, VIRGINIA, ASSESSED BY FEEDING ACTIVITY AND TROPHIC LEVEL OF MUMMICHOGS (*FUNDULUS HETEROCLITUS*)

by

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ABSTRACT

NATURALIZATION OF SALT MARSH RESTORATION SITES IN THE ELIZABETH RIVER, VIRGINIA, ASSESSED BY FEEDING ACTIVITY AND TROPHIC LEVEL OF MUMMICHOGS (*FUNDULUS HETEROCLITUS*)

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Old Dominion University, 2016
Director: Dr. Daniel M. Dauer

Recent efforts to mitigate environmental issues within the Southern Branch of the Elizabeth River, Virginia, designated a "Region of Concern" by the Chesapeake Bay Program, include several salt marsh restorations. By examining gut contents and stable isotopes values ($\delta^{13}C$ and $\delta^{15}N$) from mummichogs (*Fundulus heteroclitus*), the functional equivalency of restored salt marshes compared to natural marshes was measured. In July 2013 I collected mummichogs from three restored and three reference salt marshes in the Southern Branch. Fish were collected for gut content analysis and were analyzed for stable isotopes ($\delta^{13}C$ and $\delta^{15}N$).

I removed gut contents from 16 fish per site to measure gut fullness and identify diet composition. Muscle and liver tissue were removed from additional fish and prepared for stable isotope analysis at UC Davis. The diet composition of the restored salt marsh sites included blue-green algae as a major diet item, which was not the case in the reference marshes. The average $\delta^{13}C$ values were higher from the restored salt marshes and the average $\delta^{15}N$ values were similar between treatments. The diet composition and stable isotope analysis indicate that many of the same food items were found at the restored marshes as the reference marshes, but the restored marshes had not reached the same functional level as the reference marshes.

This thesis is dedicated to Nathan Good for his support and making a hot day in July watching *Uca pugnax* the best and to Lawrence Rudolph Tucker, who introduced me to my first freshwater macroinvertebrate and the incredible world that hides under the surface.

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First, I would like to express my deepest appreciation to Dr. Daniel Dauer for giving me the opportunity to come to Old Dominion University and work with him. He has shared his depth of knowledge and experience so that I was able to increase mine. Another person must also be thanked for giving me an understanding of benthic ecology. Anthony Rodi's patience and ability to repeat explanations many times is truly awe inspiring. Michael Lane's assistance and availability to talk over problems was invaluable as his help with map making. I would like to thank Dr. Jessica Thompson for being on my committee and teaching me the ways of the marsh. Dr. Rich Whittecar's presence on my committee has been wonderful and he has opened my eyes to hydrology.

For their willingness to get into marsh muck and collect mummichogs with me, I am forever grateful to Leah Gibala-Smith and Nathan Good. Walter Priest spent a day driving me to marshes along the Southern Branch and I thank him for the information and the fun. Thanks to Vince Barber for allowing me access through his construction site at Atlantic Wood Industries. Thank you to Phil Steadfast from Elizabeth River Terminal for letting me into the Money Point site. I appreciate the help I received from Kersten Wheeler filling out IACUC forms and meeting deadlines. Joe Rieger from the Elizabeth River Project met with me early on in developing the idea for my project and his insights were very useful. Finally, thank you to Matt Semcheski and Matt Muller for assisting me with cyanobacteria and diatom identification.

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CHAPTER 1

INTRODUCTION

Salt Marshes

Salt marsh functioning

Tidal salt marshes are dynamic coastal communities with many important ecosystem functions. Salt marshes are vegetated areas along the shores of estuaries, bays, and tidal rivers in the intertidal zone (Broome et al. 1988, Vernberg 1993). Dominate vegetation types can include grasses, sedges, and rushes, but vegetation is determined mainly by salinity, slope, and hydrology (Broome et al. 1988, Vernberg 1993). *Spartina alterniflora* is often the dominant vegetation in the Chesapeake Bay and is responsible for salt marsh platform development as water movement is slowed, detritus and sediments settle, and peat is produced (Broome et al. 1988, Mann 1988, Vernberg 1993, Pennings et al. 2012). Salt marshes produce detritus based food webs that are very complex (Pennings et al. 2012).

While *Spartina alterniflora* or other macrophytes are the dominant vegetation, the dominant food source within a marsh is actually a combination of detritus and algae (Mann 1988, Sullivan and Moncreiff 1990, Galván et al. 2008, Pennings et al. 2012). Algal detritus (benthic and planktonic) is labile and high in nitrogen (Mann 1988). Live benthic microalgae include diatoms and cyanobacteria. Benthic microalgae are abundant in salt marshes and are important food sources for invertebrates and fishes (Sullivan and Moncreiff 1990, Galván et al. 2008, Pennings et al. 2012).

There are four ways that fishes can use tidal salt marshes (Litvin and Weinstein 2003). Type I fishes are marsh residents, for example mummichogs (*Fundulus heteroclitus*), Type II fishes are marsh facultative and depend on the marsh at an early life stage (such as white perch (*Morone americana*)), Type III are transient fishes that spend some time in the marsh and move marsh products into the estuary such as the bay anchovy (*Anchoa mitchilli*), and Type IV fishes rarely enter the marsh, but benefit from marsh processes (Litvin and Weinstein 2003). The influence of a marsh within an estuary depends on the fish species using the marsh. Type III and Type IV fishes will transport nutrients and energy from the salt marsh into the estuary and beyond (Litvin and Weinstein 2003).

Salt marshes are a vital component in the flux of nutrients, biomass, and energy in estuaries, primarily through trophic interactions (Broome et al. 1988, Weinstein et al. 2005, Able et al. 2007a). The structure of salt marshes stabilizes the intertidal zone, provides habitat for a variety of organisms, and buffers shorelines from storms (Broome et al. 1988, Vernberg 1993, Peterson et al. 2008). Additionally, salt marshes have high productivity and carbon storage, which may be beneficial in slowing climate change (Vernberg 1993, Peterson et al. 2008). Tidal salt marshes are valuable for flood water storage and protecting water quality (Vernberg 1993, Peterson et al. 2008). Despite the services that salt marshes provide and benefits that humans enjoy, salt marshes have been destroyed or damaged by anthropogenic sources.

Anthropogenic threats to salt marshes include dredging, discharge of industrial products, agricultural run-off, toxic spills, and changes in hydrology caused by damming or other construction (Broome et al. 1988, Peterson et al. 2008). Oil spills are the largest

source of contaminant damage (Peterson et al. 2008). Erosion from sea level rise or coastal subsidence and storm damage can also degrade salt marshes (Broome et al. 1988). Damage to a salt marsh is usually because of a change in salinity, sedimentation, nutrients, or a combination of factors (Broome et al. 1988). The results of physical or chemical alterations to a salt marsh are losses of productivity, changes in species composition, less habitat, and destabilization of the shoreline (Broome et al. 1988). Salt marsh restoration projects are designed to combat the damage to salt marshes.

Salt marsh restoration

Salt marsh restoration projects are often the result of compensatory mitigation where the construction of a marsh is required because of the impact of coastal development (Broome et al. 1988, Able et al. 2007a, Langman et al. 2012). The purpose of restoration is to develop a salt marsh that has similar ecological functions and trophic levels as a natural marsh (Broome et al. 1988, Sacco et al. 1994, Langman et al. 2012). In order to create salt marshes, a substrate is deposited and appropriate vegetation is planted based on local natural marshes. *Spartina alterniflora* is the dominant vegetation in salt marshes along the Atlantic coast and creates intertidal marsh platforms, pools, and intertidal and subtidal creeks (Broome et al. 1988, Vernberg 1993, Able et al. 2007a). Restoration is an attempt to regain salt marsh services including providing habitat and food, acting as a storm buffer, hydrologic processing, biodiversity preservation, and carbon storage (Broome et al. 1988, Sacco et al. 1994, Able et al. 2007a, Langman et al. 2012).

A marsh restoration requires a similar elevation and tidal regime as an established marsh (Broome et al. 1988). Sand is often the substrate of choice for salt marsh restoration because sand is easy to plant in and helps prevent salinity concentrations from becoming too high because sand drains well (Broome et al. 1988). While sand is the base for many restoration projects, a salt marsh is not considered established until it has accumulated organic matter or a carbon bank, as well as having the vegetation climax species (Broome et al. 1988). A salt marsh can take 1-20 years to be considered established, depending on the metrics used for measuring recovery (Broome et al. 1988, Borja et al. 2010).

It is difficult to define the success of salt marsh restoration projects. Metrics for determining the recovery stage of restorations include plant biomass, benthic microalgae abundance, and biodiversity (Litvin and Weinstein 2003, Peterson et al. 2008). Monitoring of one or more metric of physical or biological processes is necessary for gauging the recovery of a restored salt marsh. One metric that has been used as a measure of salt marsh functional success is the mummichog.

Mummichogs as an indicator species

Mummichogs

Mummichogs (*Fundulus heteroclitus*) are often the most abundant resident fish in salt marshes along the Atlantic coast from Canada to Florida (Kneib and Stiven 1978, Teo and Able 2003a, 2003b, Able et al. 2007a). By feeding within the marsh intertidal habitat, mummichogs provide an important trophic link in estuarine systems and are generally preyed upon in the subtidal habitat, increasing the distribution of salt marsh

nutrients (Kneib et al. 1980, Kneib 1986, Currin et al. 2003, McMahon et al. 2005). Mummichogs have high site fidelity and are mainly restricted to the intertidal and subtidal habitats in salt marshes (Meredith and Lotrich 1979, McMahon et al. 2005, Skinner and Courtenay 2005, Able et al. 2007a). Mummichogs are useful for environmental monitoring programs because it is unusual for them to travel more than 600 meters up or downstream, so they are reflective of local environmental conditions (Litvin and Weinstein 2003, Teo and Able 2003b, Skinner and Courtenay 2005, Wozniak et al. 2006, Weinstein et al. 2009). The type of food and amount eaten by mummichogs is generally representative of the available resources in a salt marsh (James-Pirri et al. 2001, McMahon et al. 2005, Wozniak et al. 2006, Weinstein et al. 2009).

Mummichog diet can depend on (1) fish size, (2) feeding intertidally or subtidally, (3) marsh elevation, and (4) tidal stages and types (Kneib and Stiven 1978, Kneib 1986, Allen et al. 1994, Thompson 2015). Mummichogs are macroepibenthic predators that forage on the marsh surface, so sediment and detritus are often found in gut contents (Prinslow et al. 1974, Kneib and Stiven 1978, Allen et al. 1994, James-Pirri et al. 2001, McMahon et al. 2005). Detritus may provide some small amount of nutrition or be a source of nitrogen (Prinslow et al. 1974, Allen et al. 1994). Small crustaceans, polychaetes, nematodes, insects (larvae and adults), diatoms, algae, cyanobacteria and snails are components of mummichog diet (Prinslow et al. 1974, Kneib and Stiven 1978, Kneib 1986, Allen et al. 1994, James-Pirri et al. 2001, Litvin and Weinstein 2003, McMahon et al. 2005, Thompson 2015). Evidence of cannibalism and scavenging of other mummichogs has also been found in mummichog guts (Able et al. 2007b).

Gut content analysis

Gut content analysis (gut fullness and diet composition) of mummichogs provides information about what food is available and if feeding behavior is as expected in a healthy salt marsh. Studies of the diet components of mummichogs indicate that mummichogs are valuable as sentinel species for assessing the success of salt marsh restoration (Wozniak et al. 2006). Gut content analysis of mummichogs is considered a useful tool for evaluating salt marsh restoration naturalization by studies that indicate whether restored marshes have reached functional equivalency or not (Allen et al. 1994, James-Pirri et al. 2001). If restored salt marshes have not reached the functional equivalency of natural marshes, the gut contents indicate a lack of prey availability.

Stable isotope analysis clarifies information gained during gut content analysis. Gut content analysis provides a snapshot of what a mummichog has recently eaten, while stable isotope analysis provides an integrated measure of the fish's trophic position. Combining gut content and stable isotope analysis creates a more complete characterization of the diet of mummichogs.

Stable isotope values ($\delta^{13}C$ & $\delta^{15}N$)

Stable isotopes are isotopes (forms of the same element with different numbers of neutrons in the nucleus) that do not decay (Peterson and Fry 1987, Fry 2006). Heavy isotopes, such as ^{13}C and ^{15}N, have more neutrons than light isotopes (^{12}C and ^{14}N) (Peterson and Fry 1987, Fry 2006). Stable isotopes are measured with a mass spectrometer and the isotopic composition is expressed as δ values.

A δ value is a part per thousand difference from a standard:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N (or other isotopes) and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987, Fry 2006). So, δ values are the ratio of heavy and light isotopes in a sample. A δ value of zero, or 0‰, means that there is no difference from the standard, not that there are not any stable isotopes present (Peterson and Fry 1987, Fry 2006). An increase in the δ value is an increase in the amount of heavy isotope while a decrease would be an increase in the light isotope (Peterson and Fry 1987, Fry 2006). An increase of ~3‰ ^{15}N indicates an increase in trophic level, but $\delta^{13}C$ values change very little with trophic level, so $\delta^{13}C$ values are useful for finding carbon sources within a system (Peterson and Fry 1987, Post 2002, Fry 2006).

Stable isotope techniques can provide a measure of trophic position and track energy and mass flow through an ecosystem (Peterson and Fry 1987, Post 2002). Diet determines stable isotope composition; stable isotopes of carbon ($\delta^{13}C$) represent the source of carbon in a system (Fig. 1) and nitrogen stable isotopes ($\delta^{15}N$) reveal the path of food and trophic level (Peterson and Fry 1987, Post 2002). By comparing the feeding behavior of mummichogs from restored salt marshes to references marshes, an assessment of the functionality of the restored marsh can be made.

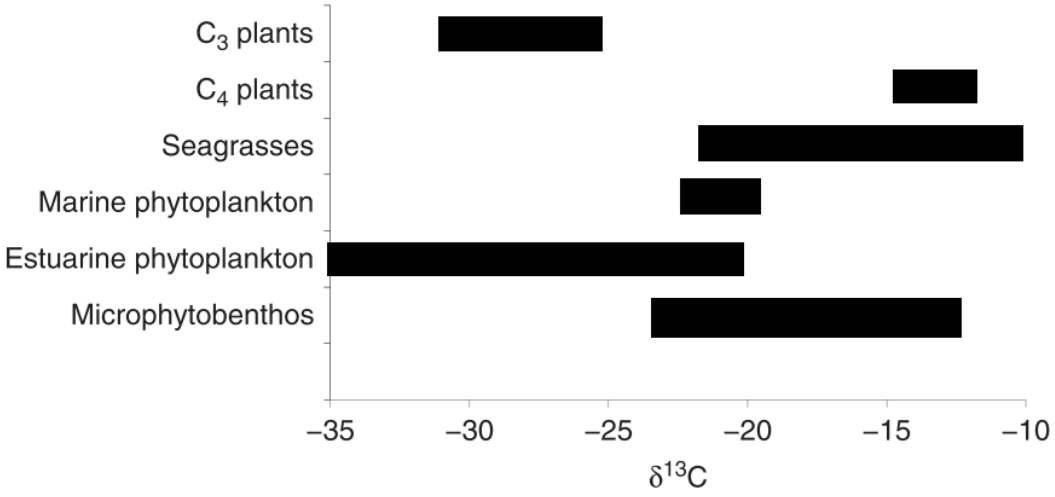

FIG. 1. Example of ranges of $\delta^{13}C$ values for selected primary producers and carbon sources within an estuary. *Juncus roemarianus* is an examples of a C_3 plant and *Spartina alterniflora* is an example of a C_4 plant (Bouillon et al. 2011).

By analyzing the $\delta^{13}C$ and $\delta^{15}N$ values in both liver and muscle from mummichogs, I was able to determine short and long term feeding sources and trophic levels (Peterson and Fry 1987, Logan et al. 2006, Haas et al. 2009). Liver tissue has a higher turnover rate than muscle tissue, so liver tissue reflects more recent diet while muscle tissue reflects long-term diet (Logan et al. 2006, Haas et al. 2009).

A study by Wozniak and Roman (2006) compared $\delta^{13}C$ values in mummichog muscle from fish in restored marshes to fish in reference marshes and found that $\delta^{13}C$ values were more enriched in the reference marsh. The more similar the restored marsh was to the reference marsh, the greater the $\delta^{13}C$ values were for the restored marsh mummichogs, indicating that a food web change occurred as restored marshes became more functionally equivalent to references marshes (Wozniak et al. 2006).

In a natural salt marsh $\delta^{15}N$ values increase with mummichog total length, indicating an increase in trophic level with ontogeny (Post 2002, Currin et al. 2003). An adult mummichog in a healthy salt marsh should be feeding at approximately two trophic levels above primary producers (Currin et al. 2003). As the ratio of carbon ($\delta^{13}C$) does not change substantially through a food web $\delta^{13}C$ values in a natural salt marsh should reflect the dominant primary producer (Post 2002, McMahon et al. 2005, Logan et al. 2006). A restored salt marsh that is not similar in functional equivalency to a natural marsh will have $\delta^{15}N$ values that indicate mummichogs feeding at a trophic level less than two above primary producers and $\delta^{13}C$ values possibly indicating a different primary carbon source than in natural marshes (Peterson and Fry 1987, Currin et al. 2003, Haas et al. 2009). By using gut content analysis and stable isotope analysis in tandem in my study, the current available food sources and possible past food sources were determined.

Elizabeth River

A number of restored marshes have been created in the Elizabeth River, Virginia, within the past decade, making the river an ideal location to compare restored salt marshes to natural salt marshes. The Elizabeth River has three branches: the Eastern Branch, the Western Branch, and the Southern Branch. For the Hampton Roads region of Virginia, the Elizabeth River is an important shipping channel and is dredged to maintain depths necessary for ships. The Elizabeth River watershed is heavily industrialized and the river has been contaminated by heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Dauer 1993, Mitra et al. 1999, Dauer and Llansó 2003, Conrad and Chisholm-Brause 2004, Conrad et al. 2007). The Southern Branch of the Elizabeth River is one of

the most industrialized areas in the lower Chesapeake Bay and has the highest concentrations of sediments contaminants in the Elizabeth River (Hawthorne and Dauer 1983, Dauer 1993, Conrad and Chisholm-Brause 2004, Conrad et al. 2007, Webb 2014).

Fundulus heteroclitus from the Elizabeth River are exposed to PAHs throughout their life cycles. PAHs are HOCs (hydrophobic organic contaminants) that are often associated with sediments (Mitra et al. 1999). Many PAHs are carcinogenic and mutagenic and are on the US EPA Priority Pollutant List (Mitra et al. 1999, Jung et al. 2011). The benthic communities of the Southern Branch, which includes many organisms preyed on by mummichogs, are degraded because of sediment contamination (Dauer and Llansó 2003). Mummichogs from the Elizabeth River have a heritable tolerance to PAH exposure compared to fish from uncontaminated systems, but PAHs still cause DNA damage, hepatic lesions, and tumor growths (Vogelbein et al. 1990, Ownby et al. 2002, Jung et al. 2011). Heavy metal contamination can cause decreased prey capture ability and increased mortality from predation in mummichogs (Smith and Weis 1997, Weis et al. 2003). Because of the contamination in the Elizabeth River, natural salt marshes in the Southern Branch may not be the functional equivalent of salt marshes in uncontaminated river systems, but within the Southern Branch they are comparable.

Objectives

After a salt marsh is constructed, it is necessary to monitor various functions in order to assess if the construction was successful. Because *Fundulus heteroclitus* have high site fidelity and a diet that is reflective of available small prey, they are an ideal

indicator species for measuring salt marsh functions (Kneib 1986, Allen et al. 1994, James-Pirri et al. 2001, McMahon et al. 2005). The objectives of my study were to determine if there were differences between mummichogs from restored salt marshes and reference salt marshes in primary sources of carbon, trophic level and diet. I tested the hypothesis that there is no difference in carbon stable isotopic values, nitrogen stable isotopic values, gut fullness, and diet components of *Fundulus heteroclitus* between restored marshes and reference sites.

CHAPTER 2

MATERIALS AND METHODS

Study sites

To study the differences between restored salt marshes and mature, naturally developed marshes, I chose six salt marsh sites within the Southern Branch of the Elizabeth River, a tributary of the Chesapeake Bay (Fig. 2). The Elizabeth River has been the focus of many remediation projects and I chose three young restoration sites that are located in heavily contaminated areas for my research (Fig. 2). Three mature reference salt marshes were chosen upstream from contamination based on the advice of Walter Priest, Habitat Restoration Specialist at NOAA Restoration Center (Fig. 2).

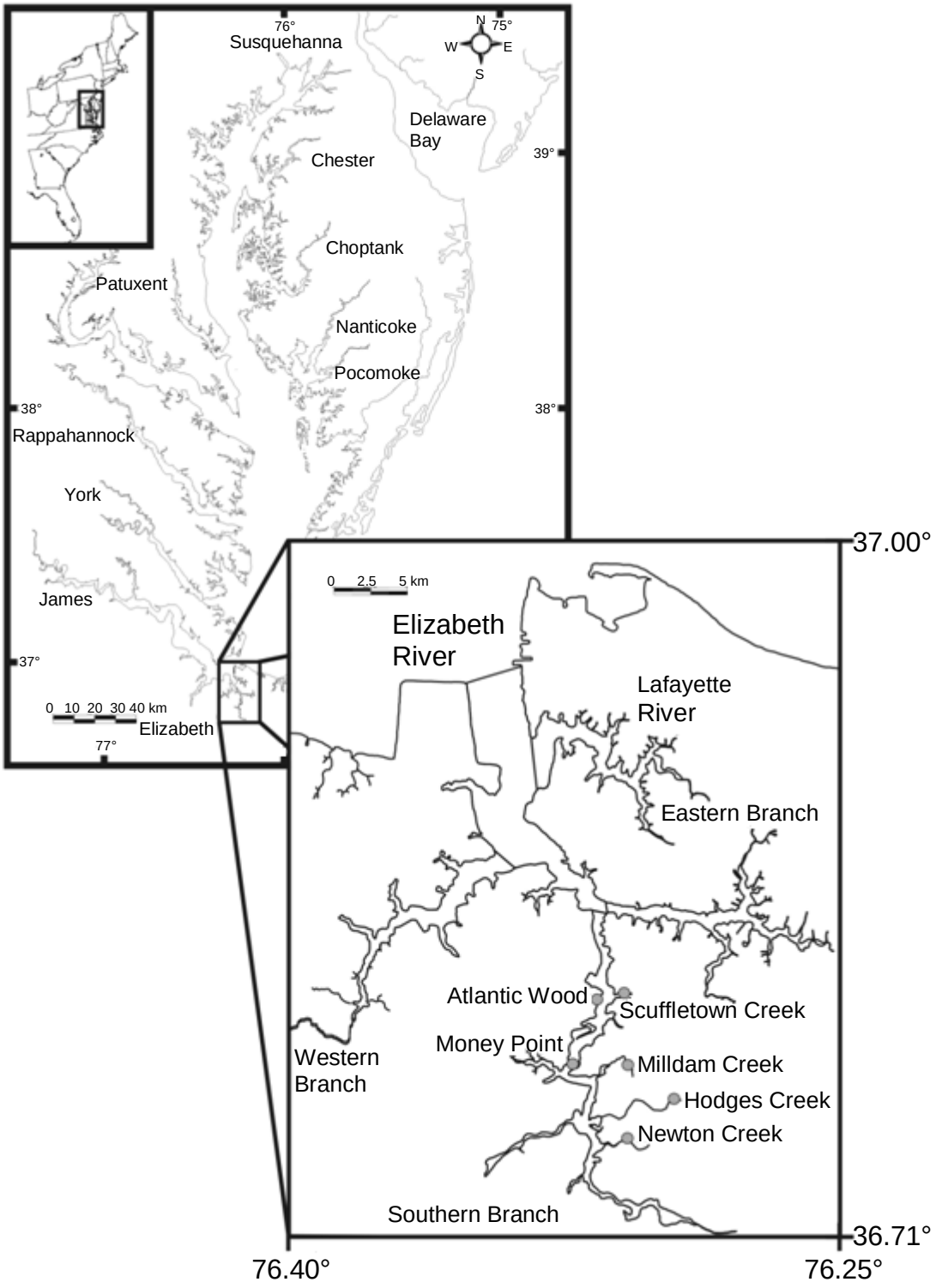

FIG. 2. Map of Chesapeake Bay watershed with box indicating location of the Elizabeth River and insert with locations of study sites. Restored salt marshes are Atlantic Wood, Scuffletown Creek, and Money Point. Reference salt marshes are Milldam Creek, Newton Creek, and Hodges Creek. Map author: Mike Lane.

The Atlantic Wood Industries property (36° 48’25” N, 76° 17’40” W) is an EPA superfund site. The restored salt marsh was built in 2004 and is approximately 0.5 hectares. While I was collecting from the Atlantic Wood marsh, there was ongoing construction work next to the marsh. The restored marsh is on the main stem of the Southern Branch and while I was sampling, the marsh had floating blocks across the opening to the main stem, presumably to cut down on litter. The Atlantic Wood marsh had the most garbage (plastic bags, cans, etc.) within the marsh out of all the study sites. The Scuffletown Creek restored salt marsh (36° 48’35” N, 76° 17’01” W) was built in 2010 and is approximately 0.3 hectares. The Scuffletown site is approximately 0.3 km away from the main stem of the Southern Branch. Money Point (36° 46’57” N, 76° 18’08” W) has been the site of sediment remediation as well as the construction of an approximately 2 hectare salt marsh in 2008. A rock sill was built along the edge of the marsh with opening to the main stem of the Southern Branch.

The reference marsh chosen in Milldam Creek (36° 47’02” N, 76° 16’59” W) is approximately 1.3 hectares and approximately 1.3 km away from the Southern Branch main stem. Milldam Creek is the closest reference site to the restored sites. The Newton Creek salt marsh site (36° 46’11” N, 76° 17’17” W) is approximately 0.6 hectares and 1 km away from the Southern Branch main stem. The reference site that is the farthest from the restored sites is located in Hodges Creek. The Hodges Creek salt marsh (36° 45’36” N, 76° 17’14” W) is approximately 3.2 hectares and approximately 0.5 km away from the main stem of the Southern Branch.

Sample Collection

Prior to my collecting any *Fundulus heteroclitus*, my methods were reviewed and approved by the Institutional Animal Care and Use Committee of Old Dominion University (IBC Approval Number 13-009).

In July, 2013, I collected mummichogs ≥ 40 mm using unbaited minnow traps. During the ebbing and flooding tide, the minnow traps were set in the salt marsh so that water was flowing through. Traps were emptied every 15 minutes until a sufficient number of mummichogs were collected from the intertidal and subtidal zone. Research has indicated that mummichogs feed on different items in the intertidal and subtidal zone (Thompson 2015). Occasionally a seine net was also used if the minnow traps were not catching any fish, typically due to logistical difficulties with trap placement.

From each site, eight fish were collected from the intertidal zone and eight fish were collected from the subtidal zone for gut content analysis. Individuals for gut content analysis were euthanized with a lethal dose of MS-222 (tricaine methanesulfonate). Preservation of the gut contents was accomplished by inserting a 1 cc syringe in the cloaca of the fish and injecting a 10% buffered formalin solution. Individuals were then placed in a five-gallon bucket filled with 10% buffered formalin solution. Fish for stable isotope analysis were collected from each site after the number for fish for gut content analysis were collected. After 15 fish were collected from each site for stable isotope analysis, the mummichogs were immediately placed on ice for transport to the lab, where they were frozen at -20°C.

Laboratory Analysis

Gut Content Analysis

The sex, total length (TL) in millimeters, and wet weight (± 0.01 g) (weight of fish blotted dry) were recorded for each individual. I completed gut content analysis based on Hylsop's (1980) methods for Gut Fullness Indices and subjective measurements of prey item composition for small fish. Gut Fullness Indices are the percentage of body weight that is from the contents of the guts. Mummichogs do not have a stomach, but rather a gut that can be divided into three sections. Sections I and II of the digestive tract were removed, blotted dry, and weighed to the nearest 0.001 g (Babkin and Bowie 1928). The third section contains almost completely digested food material, so it is not useful for food item identification. The contents of sections I and II of the digestive tract were removed and the gut was reweighed. The gut content weight was determined by the difference between the full gut and the empty gut. I divided gut content weight by wet weight and multiplied by 100 to calculate a Gut Fullness Index for each fish (Hyslop 1980).

The subjective method described by Hyslop (1980) is the determination of the approximate percent out of the total gut contents that is filled by each food item. The gut contents were removed and identified to preselected categories using a dissecting scope. The categories were ranked based on presence within the gut as part of the subjective method (3= >50%, 2= 10-50%, 1= <10%, 0= absent) (Allen et al. 1994, James-Pirri et al. 2001, Thompson 2015).

Stable Isotope Analysis

I thawed mummichogs collected for stable isotope analysis and removed the whole liver and an entire muscle filet for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. Samples were dried in an oven for 24 hours. I powdered samples with a mortar and pestle until homogenous before placing each sample into a tin capsule. I organized samples into 96-well trays for shipment to University of California, Davis Stable Isotope Facility.

At the UC Davis Stable Isotope Facility, samples were analyzed for ^{13}C and ^{15}N by isotope ratio mass spectrometry using a PDZ ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 mass spectrometer. The samples were compared to laboratory standards calibrated against National Institute of Standards and Technology (NIST) Standard Reference Materials. The UC Davis Stable Isotope Facility delivered values for stable isotopes in delta values (δ) expressed relative to international standards. Vienna PeeDee Belemnnite (V-PDB) is the standard for carbon and air (N_2) is the standard for nitrogen.

Statistical Analyses

I used one-factor fixed analysis of variances (ANOVAs) to test for differences in fish length (mm), Gut Fullness Indices percentages and stable isotope values. I tested for assumption of normality with Shapiro-Wilk test and assumption of homogeneity of variance with Levene's test. Statistic analyses were run in SPSS 21. When an ANOVA indicated differences between sites, Tukey's honest significant difference (HSD) post-hoc tests were used to identify differences. A Chi-square test of the frequency of diet

components being >50% of gut contents was used to determine significant differences in diet components between restored and references marshes.

CHAPTER 3

RESULTS

Gut Fullness Indices

Fish length is a factor impacting gut fullness and the results of a one-way ANOVA with a log10 transformation and Tukey's post-hoc indicated a significant difference in fish length between Newton Creek and Atlantic Wood ($F = 2.664$; 5, 90; $P = 0.027$), but no significant difference in fish length between any other sites. Mummichogs from the three restored sites had higher Gut Fullness Indices averages than the mummichogs collected from the three reference sites (Fig. 3). Restored site Gut Fullness Indices averages were between 3.2% and 3.5%, while reference site Gut Fullness Indices percentage were between 1.3% and 2.4%. Newton Creek had the highest Gut Fullness Indices percentage averages from the reference sites (Fig. 3).

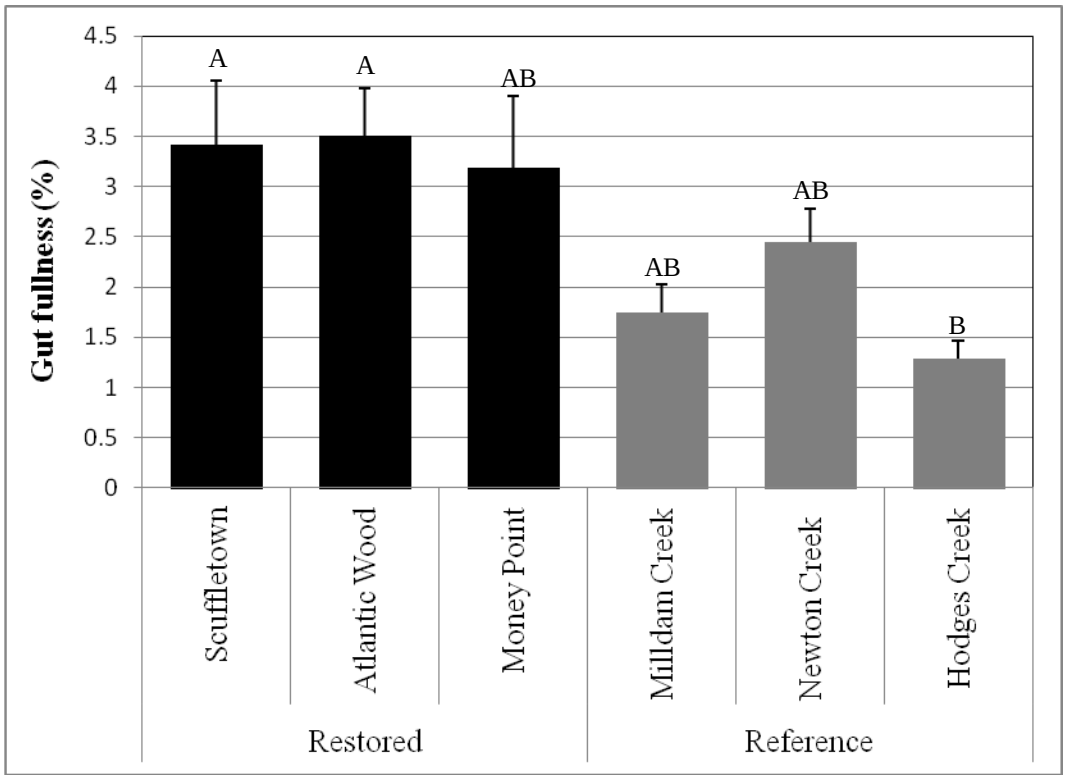

FIG. 3. Gut Fullness Indices (%) of mummichogs collected from three restored salt marshes and three reference salt marshes from the Southern Branch of the Elizabeth River, VA. Error bars are standard error of the mean (SEM). Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Fish from restored marshes had significantly fuller guts than fish from reference marshes ($F = 13.12$; $df = 1, 4$; $P = 0.022$) based one-way ANOVA with an angular transformation (unable to pass Homogeniety of Variance test (0.010 sig Levene). When I ran a one-way ANOVA with an angular transformation to test for differences among sites, there were significant differences among sites ($F = 4.15$; $df = 5, 90$; $P = 0.002$). The results of a Tukey's post hoc had significant differences between Atlantic Wood and Hodges Creek as well as Scuffletown Creek and Hodges Creek.

Diet Components

The diet components from 96 mummichog guts were identified. If the diet component was present in >10% of the guts from restored or reference marshes, I considered it a major diet component. I identified 19 major diet items and 7 minor diet items (Table 1). Detritus from dead plant material were present in almost every fish to varying degrees (Table 1). Fish from restored sites also had filamentous cyanobacteria enmeshed with detrital material in the guts. I call cyanobacteria with detritus "cyanobacteria detrital complex" (CDC). The cyanobacteria dominated the complex. The fish from reference sites did not have any cyanobacteria detritus complexes in the gut contents (Fig. 4; Table 1). Crabs were more common in fish from reference sites (Fig. 5). A Chi-square test of the frequency of diet components being >50% of the gut contents between restored and reference sites showed that there were significant differences between restored and reference sites in the frequency of cyanobacteria detrital complex ($P < 0.0001$), crabs ($P = 0.0002$), eggs ($P = 0.007$), turbellaria ($P = 0.003$), and ostracods ($P = 0.011$). Diatoms were another major diet item that was significantly more common ($P > 0.0001$) in restoration site guts (Fig. 4). Scales were present at every site and are likely contamination from processing (Fig. 4).

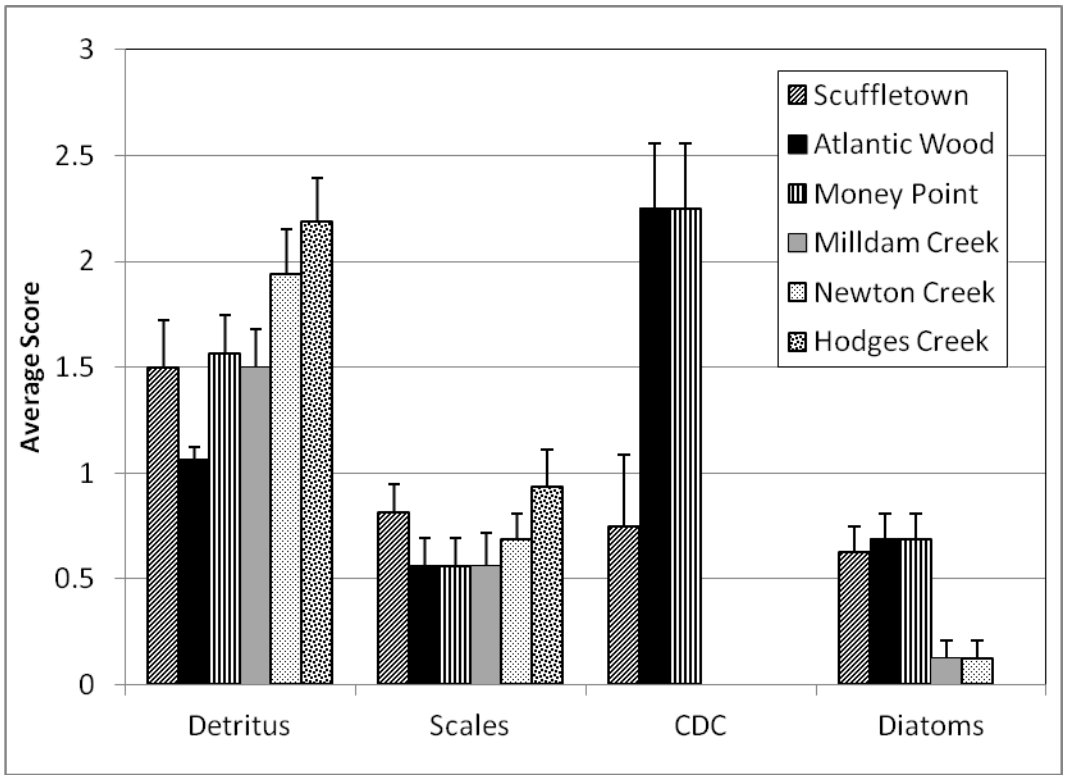

FIG. 4. Selection of major (present in >10% of fish from site) diet components of mummichogs collected from three restored salt marshes (Scuffletown Creek, Atlantic Wood, and Money Point) and three reference salt marshes (Milldam Creek, Newton Creek, and Hodges Creek) from the Southern Branch of the Elizabeth River, VA. Average score within guts for each diet component: 3 = abundant (>50%), 2 = common (10-50%), 1 = present (<10%), and 0 = absent. CDC is cyanobacteria detrial complex. Error bars are standard error of the mean (SEM).

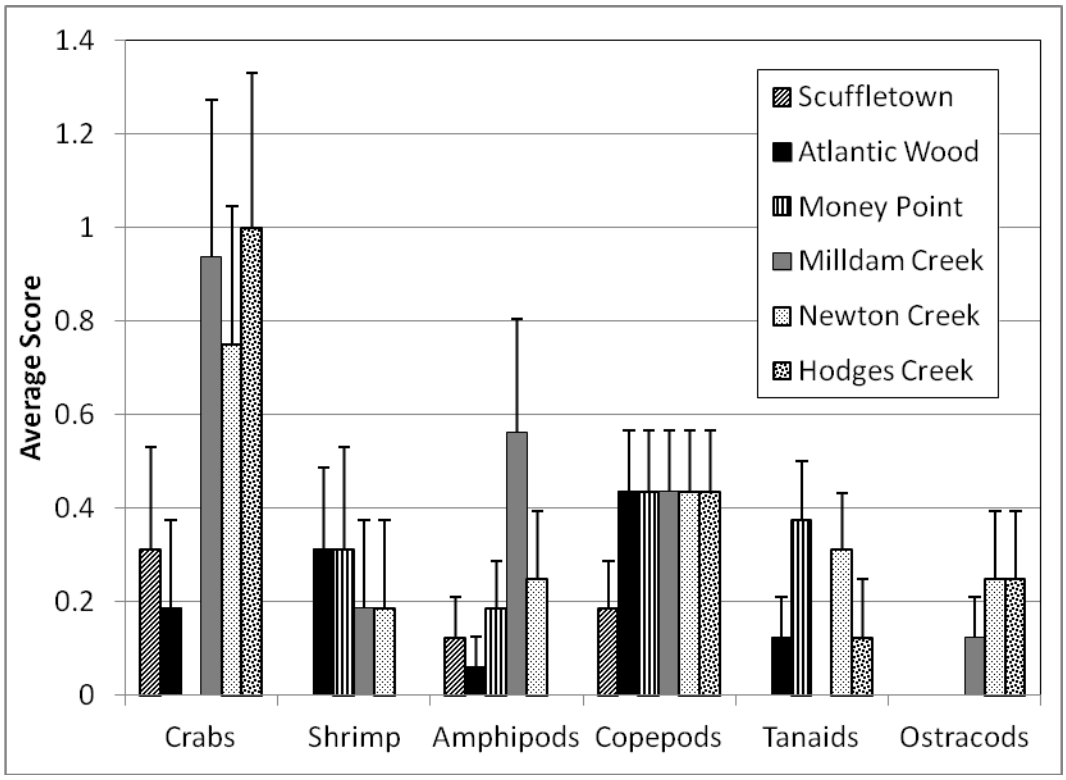

FIG. 5. Major (present in >10% of fish from site) crustacean diet components of mummichogs collected from three restored salt marshes (Scuffletown Creek, Atlantic Wood, and Money Point) and three reference salt marshes (Milldam Creek, Newton Creek, and Hodges Creek) from the Southern Branch of the Elizabeth River, VA. Average score within guts for each diet component: 3 = abundant (>50%), 2 = common (10-50%), 1 = present (<10%), and 0 = absent. Error bars are standard error of the mean (SEM).

TABLE 1. Percentage of mummichog collected from sites in the Southern Branch of the Elizabeth River, VA, with guts containing diet components and mean gut fullness (GFI ± SEM) per site. Number of fish examined per site (*n*) was 16. In parentheses for each major diet component is the percentage of fish from the site that the item was >50% of the gut contents. UOM stands for Unidentified Organic Matter.

	Restoration Sites			**Reference Sites**		
	Scuffletown Creek	**Atlantic Wood**	**Money Point**	**Milldam Creek**	**Newton Creek**	**Hodges Creek**
GFI	3.4 ± 0.6	3.5 ± 0.5	3.2 ± 0.7	1.7 ± 0.3	2.4 ± 0.3	1.3 ± 0.2
Major Components (≥10% within a treatment)						
Detritus	93.8 (18.8)	100 (0)	100 (12.5)	100 (12.5)	100 (43.8)	100 (31.3)
Fish Scales	75 (0)	56.3 (0)	56.3 (0)	50 (0)	68.8 (0)	75 (0)
Cyanobacteria detrital complex	25 (25)	81.3 (68.8)	56.3 (56.3)	0 (0)	0 (0)	0 (0)
Diatoms	62.5 (0)	68.8 (0)	43.8 (0)	12.5 (0)	12.5 (0)	0 (0)
Polychaetes	43.8 (6.3)	31.3 (0)	43.8 (6.3)	50 (0)	43.8 (12.5)	31.3(18.8)
Crab	12.5 (6.3)	6.3 (6.3)	0 (0)	37.5 (25)	31.3 (12.5)	43.8 (25)
Copepods	18.8 (0)	43.3 (0)	43.8 (0)	43.8 (0)	18.8 (0)	43.8 (0)
Eggs	18.8 (6.3)	6.3 (0)	6.3 (0)	50 (18.8)	31.3 (0)	18.8 (6.3)
Insects	18.8 (0)	18.8 (6.3)	12.5 (6.3)	6.3 (0)	18.8 (0)	6.3 (0)
Aquatic insect larvae	31.8 (0)	6.3 (0)	12.5 (0)	18.8 (0)	37.5 (0)	12.5 (0)
Amphipods	12.5 (0)	6.3 (0)	18.8 (0)	31.3 (6.3)	18.8 (0)	0 (0)
Shrimp	0 (0)	18.8 (0)	12.5 (6.3)	6.3 (6.3)	6.3 (6.3)	0 (0)
Nematodes	31.3 (0)	6.3 (0)	0 (0)	37.5 (0)	6.3 (0)	6.3 (0)
Tanaids	0 (0)	12.5 (0)	37.5 (0)	0 (0)	31.3 (0)	6.3 (0)
Turbellaria	0 (0)	18.8 (0)	0 (0)	25 (0)	18.8 (0)	43.8 (0)
Filamentous algae	18.8 (0)	18.8 (0)	6.3 (0)	0 (0)	31.3 (0)	0 (0)
Foraminifera	31.3 (0)	25 (0)	0 (0)	12.5 (0)	0 (0)	6.3 (0)
Mites	31.3 (0)	0 (0)	12.5 (0)	12.5 (0)	0 (0)	0 (0)
Ostracods	0 (0)	0 (0)	0 (0)	12.5 (0)	6.3 (0)	18.8 (0)
Minor Components						
Algal Detrital Complex	18.8	0	0	0	0	0
Cyanobacteria	25	0	0	0	0	0
UOM	6.3	0	0	0	12.5	6.3
Spiders	0	6.3	6.3	6.3	6.3	0

TABLE 1. Continued

Bivalves	0	0	6.25	0	0	0
Gastropods	0	0	0	6.25	6.25	0
Isopods	0	0	0	0	12.5	0

Stable Isotope Values

Stable Isotope Values from Muscle Samples

I collected a total of 90 mummichogs for stable isotope analysis and muscle samples from all fish were analyzed. Two reference sites muscle tissue samples were depleted in $\delta^{13}C$ compared to three restored sites and the Newton Creek reference site (Fig. 6; Table 2). A change in trophic position is a difference of ~3 $\delta^{15}N$ value. All sites appeared to have fish that were feeding at the same trophic level (Fig. 6). Sites can have the same trophic position because there is not a large enough difference in $\delta^{15}N$ values, but still be significantly different because there is a P value less than 0.05. There were significant differences in $\delta^{13}C$ muscle values between restored and reference sites ($F= 195.7$; $df = 1, 88$; $P < 0.0001$) based on a one-way ANOVA with a rank transformation. A one-way ANOVA with a rank transformation with a Tukey's post hoc ($F = 101.8$; $df = 5, 84$; $P < 0.0001$) testing $\delta^{13}C$ muscle differences between sites indicated that Hodges Creek and Milldam Creek (both reference sites) were significantly different from the four other sites (Fig. 7). Newton Creek (reference) was significantly different from all other sites, as was Atlantic Wood (restored). Scuffletown Creek and Money Point (restored) were not significantly different from each other (Fig. 7).

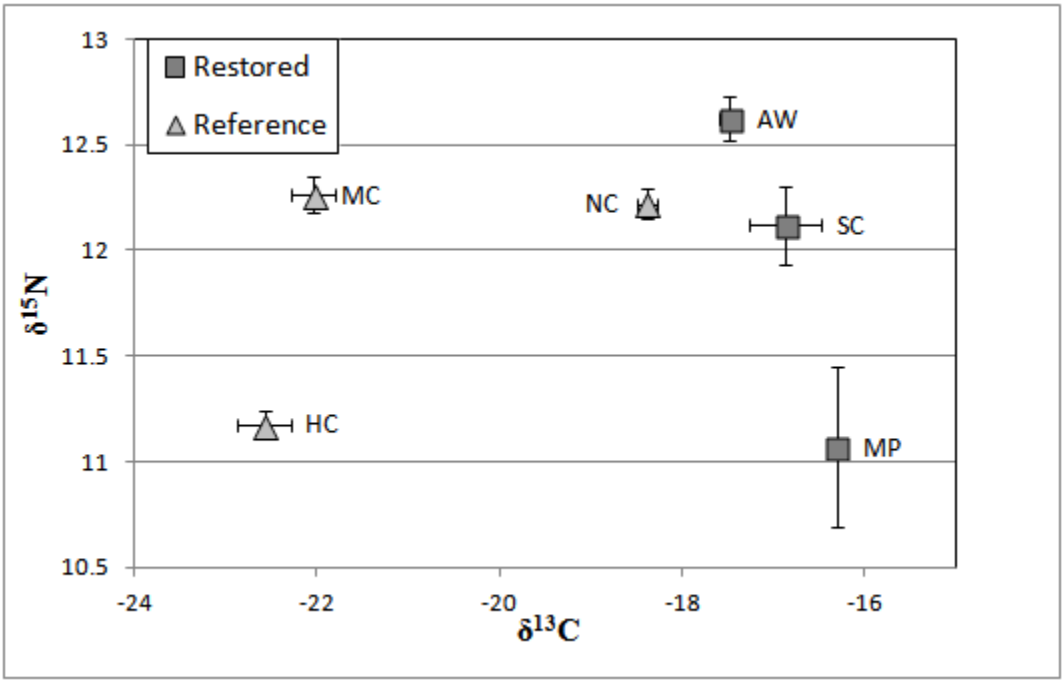

FIG. 6. Average stable isotope values (‰) of nitrogen and carbon from mummichog muscle tissue for each restored site (AW, SC, & MP) and reference site (MC, NC, & HC) from the Elizabeth River. Error bars are standard error of the mean (SEM).

TABLE 2. Average muscle $\delta^{13}C$ and $\delta^{15}N$ values with standard error of the mean (SEM) from mummichogs collected in the Southern Branch of the Elizabeth River. AW-Atlantic Wood, MP-Money Point, SC-Scuffletown Creek, MC-Milldam Creek, NC-Newton Creek, HC-Hodges Creek. Number of fish muscle tissue sampled from (*n*) for each site was 15.

	Muscle Stable Isotope Averages					
	Restored			Reference		
	AW	MP	SC	MC	NC	HC
$\delta^{13}C \pm$ SEM	-17.49 ± 0.11	-16.31 ± 0.10	-16.87 ± 0.39	-22.04 ± 0.24	-18.39 ± 0.11	-22.58 ± 0.30
$\delta^{15}N \pm$ SEM	12.62 ± 0.11	11.07 ± 0.38	12.12 ± 0.18	12.26 ± 0.09	12.22 ± 0.07	11.18 ± 0.07

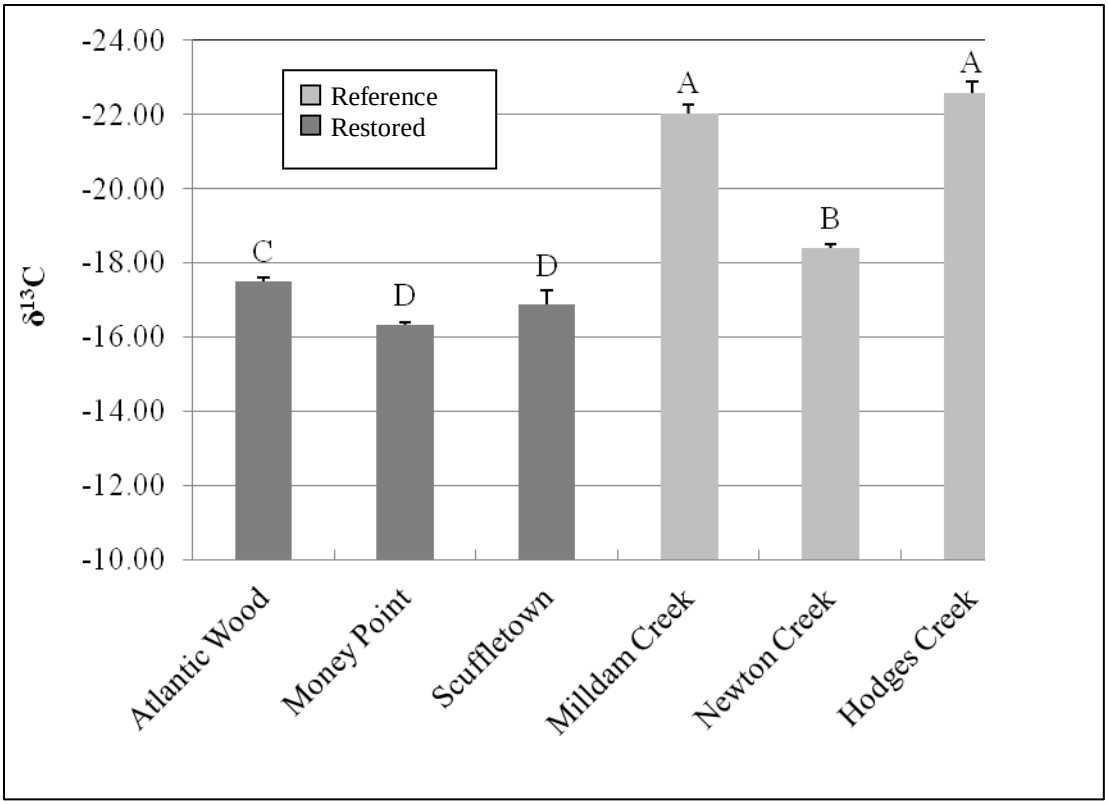

FIG. 7. Mean muscle tissue $\delta^{13}C$ values ± SEM of mummichogs collected from three restored salt marshes and three reference salt marshes from the Southern Branch of the Elizabeth River, VA. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

There were not any significant differences in muscle $\delta^{15}N$ values between restored and reference treatments ($F = 1.7$; $df = 1, 88$; $P = 0.194$) based on an ANOVA with a rank transformation. Hodges Creek (reference site) and Money Point (restored site) were significantly different from the four other sites in muscle $\delta^{15}N$ values ($F = 13.8$; $df = 5, 84$; $P < 0.0001$) as indicated by a one-way ANOVA with a rank transformation testing for differences among sites (Fig. 8).

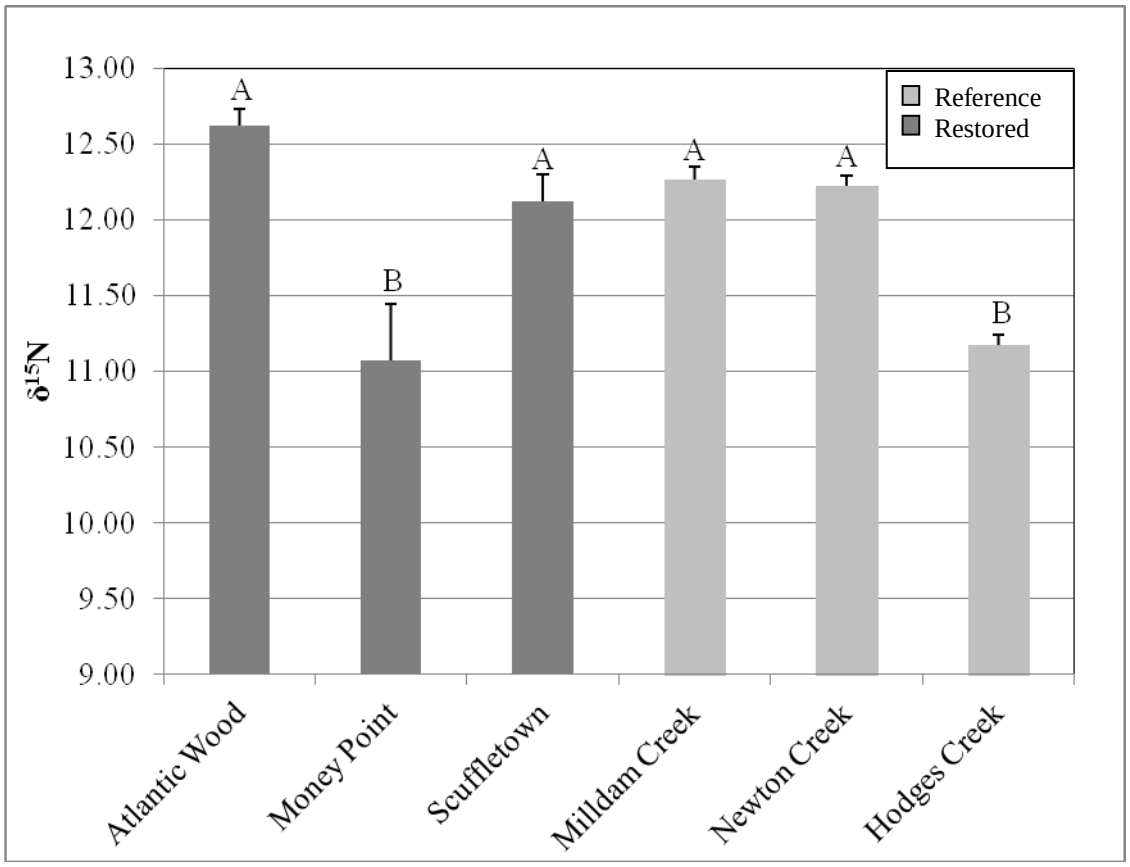

FIG. 8. Mean muscle tissue $\delta^{15}N$ values ± SEM of mummichogs collected from three restored salt marshes and three reference salt marshes from the Southern Branch of the Elizabeth River, VA. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

Stable Isotope Values from Liver Samples

Out of the 90 fish collected for stable isotope analysis, I was able to collect liver tissue samples from 73 fish (Table 4). Livers from reference sites were more depleted in $\delta^{13}C$ than livers from restored sites (Fig. 9; Table 3). $\delta^{15}N$ liver values were similar, with Atlantic Wood liver tissue having the highest $\delta^{15}N$ value (11.23 ± 0.17) and Hodges Creek having the lowest $\delta^{15}N$ value (9.37 ± 0.18) (Fig. 9; Table 3).

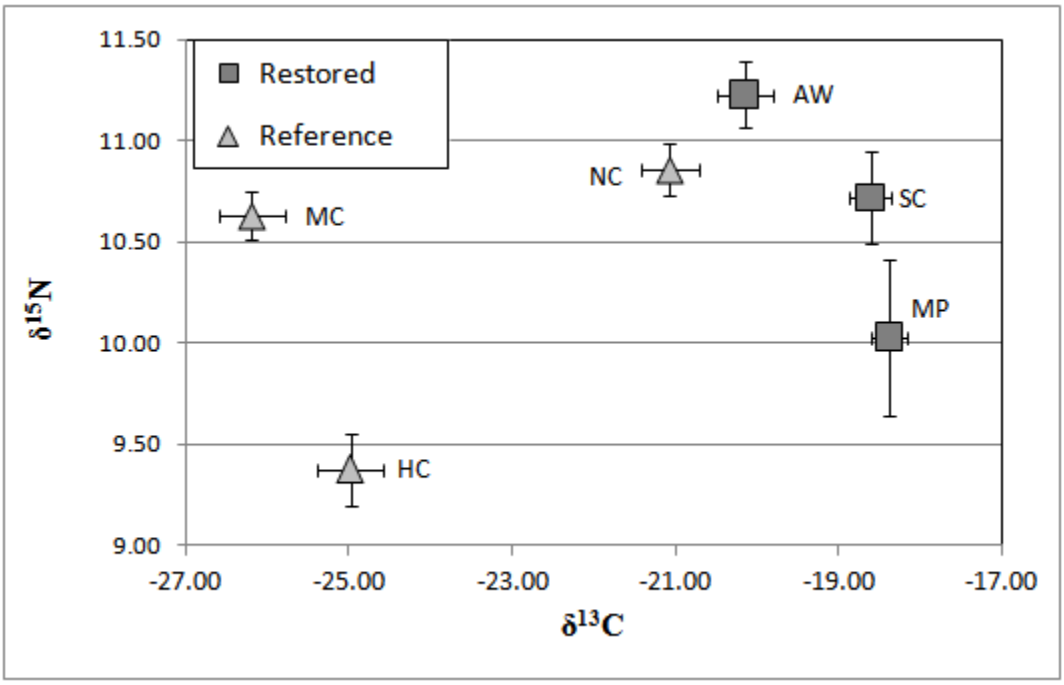

FIG. 9. Average stable isotope values (‰) of nitrogen and carbon from mummichog liver tissue for each restored site (AW, SC, & MP) and reference site (MC, NC, & HC) from the Elizabeth River. Error bars are standard error of the mean (SEM).

TABLE 3. Average liver $\delta^{13}C$ and $\delta^{15}N$ values with standard error of the mean (SEM) and number of fish liver tissue sampled from (*n*) for each site (AW-Atlantic Wood, MP-Money Point, SC-Scuffletown Creek, MC-Milldam Creek, NC-Newton Creek, HC-Hodges Creek), in the Southern Branch of the Elizabeth River.

	Liver Stable Isotope Averages					
	Restored			Reference		
	AW	MP	SC	MC	NC	HC
n	12	12	11	14	12	12
$\delta^{13}C \pm$ SEM	-20.15 ± 0.34	-18.38 ± 0.21	-18.61 ± 0.25	-26.19 ± 0.41	-21.06 ± 0.35	-24.98 ± 0.41
$\delta^{15}N \pm$ SEM	11.23 ± 0.17	10.03 ± 0.39	10.72 ± 0.23	10.63 ± 0.12	10.86 ± 0.13	9.37 ± 0.18

There was a significant difference between the liver $\delta^{13}C$ values from restored sites and reference sites based on a one-way ANOVA with a rank transformation

(F = 20.7; df = 1, 71; P < 0.0001). A one-way ANOVA with a Tukey's post hoc test testing for differences among marsh sites (F = 94.1; df = 5, 67; P < 0.0001) indicated that Atlantic Wood and Newton Creek liver $\delta^{13}C$ values were not significantly different from each other and were significantly different from the other sites; Scuffletown and Money Point liver $\delta^{13}C$ values were not significantly different from each other and were significantly different from the other sites; and Hodges Creek and Milldam Creek liver $\delta^{13}C$ values were not significantly different from each other and were significantly different from the other sites (Fig. 10).

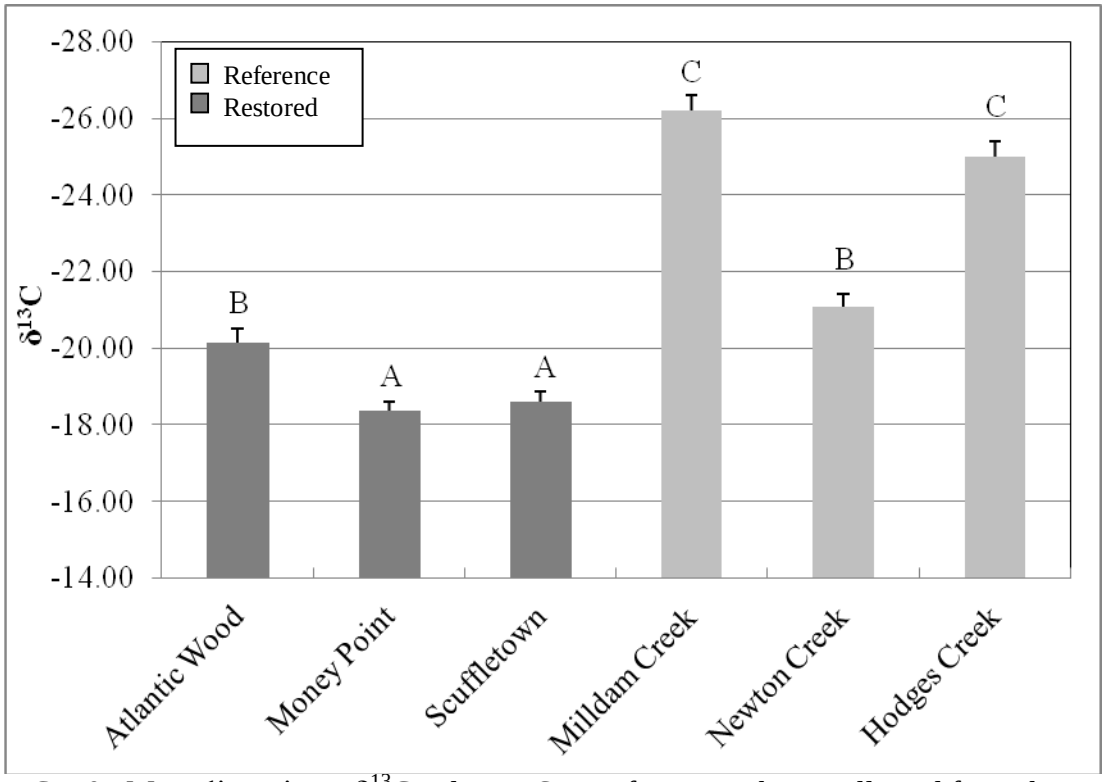

FIG. 10. Mean liver tissue $\delta^{13}C$ values ± SEM of mummichogs collected from three restored salt marshes and three reference salt marshes from the Southern Branch of the Elizabeth River, VA. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

There was a significant difference between restored and reference treatments for liver tissue $\delta^{15}N$ values ($F = 04.0$; $df = 1, 71$; $P = 0.050$) based on a one-way ANOVA with a power square transformation. Liver $\delta^{15}N$ values between Atantic Wood, Newton Creek, Scuffletown Creek, and Milldam Creek were not significantly different from each other ($F = 9.6$; $df = 5,67$; $P < 0.0001$) as indicated by a one-way ANOVA with a rank transformation and a Tukey's post hoc test. Hodges Creek $\delta^{15}N$ values for liver were significantly different from Atantic Wood, Newton Creek, Scuffletown Creek, and Milldam Creek, but not significantly different from Money Point. Money Point liver $\delta^{15}N$ values were significantly different from Atlantic Wood (Fig. 11).

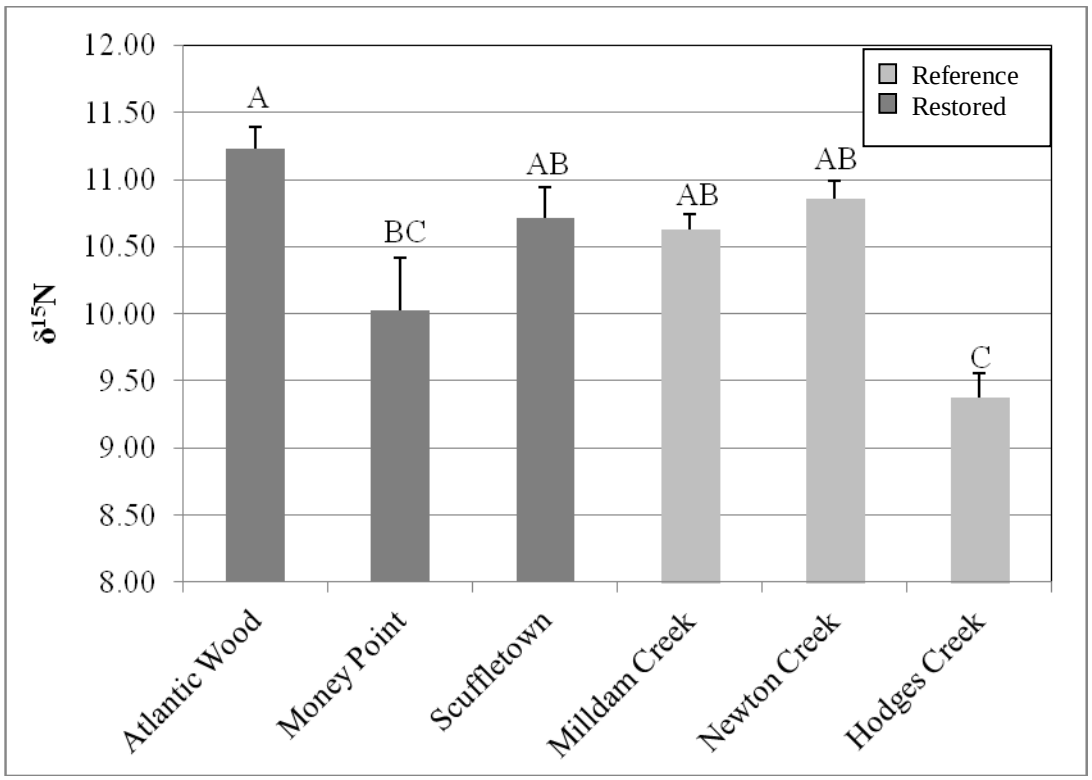

FIG. 11. Mean liver tissue $\delta^{15}N$ values ± SEM of mummichogs collected from three restored salt marshes and three reference salt marshes from the Southern Branch of the Elizabeth River, VA. Means with different letters are significantly different (Tukey's HSD, $p<0.05$).

CHAPTER 4

DISCUSSION

Implications of Gut Fullness Indices and Diet Components for Restoration Success

There was a striking difference between the restored salt and reference marshes Gut Fullness Indices. Fish from the restored salt marshes had significantly fuller guts than fish from references marshes. Mummichogs that feed in the intertidal zone have greater gut fullness than fish feeding in the subtidal zone, but that should not have influenced my study because I attempted to collect fish that had been feeding in both zones (Allen et al. 1994, Thompson 2015). Fish size is also a factor in mummichog gut fullness. When feeding in the intertidal zone, small fish (40-60 mm total length) have a greater gut fullness than larger fish, unless the large fish has consumed a large prey item (e.g., crab and shrimp) (Thompson 2015). With the exception of Atlantic Wood (72.9 mm total length), all average fish lengths (mm) were within the range of 60-70 mm, so length was not likely a major cause of gut fullness. However, the length (mm) of fish from Atlantic Wood was significantly greater than fish from Newton Creek. The average Gut Fullness Indices of Newton Creek mummichogs was the closest to the restored sites values out of the three reference sites. Fish length may explain why Newton Creek fish had the highest gut fullness values out of the reference sites, but it does not explain why the fish from the three restored salt marshes had the highest average Gut Fullness Indices out of all six sites sampled. Diet of the mummichogs may explain differences in gut fullness.

Guts of fish from restored salt marshes were often packed with cyanobacteria strands. When cyanobacteria detrital complex was present in fish, it was usually given a

score of 3, indicating that the diet item comprised greater than 50% of the gut contents. The presence of cyanobacteria is the most likely explanation for the higher Gut Fullness Indices from restored sites. Filamentous cyanobacteria form mats on the marsh surface and can be easily ingested by mummichogs attempting to forage (Prinslow et al. 1974, Kneib et al. 1980, Kneib 1986, Peterson et al. 1986, Zheng et al. 2004). Cyanobacteria are not uncommon in recently restored salt marshes and may actually be an important source of nitrogen for a developing marsh (Currin et al. 1995, 1996, Piehler et al. 1998, Zheng et al. 2004). Conditions that are favorable to cyanobacteria growth include greater irradiance and water temperature (Currin et al. 1996, Watermann and Hillebrand 1999). The substrate in planted salt marshes is predominately sand and there is little plant cover to provide shade so there is higher irradiance and water temperature than in a mature marsh. As plant detritus in a restored salt marsh sediment and plant cover increases over time, the salt marsh will be less likely to have cyanobacteria mats (Currin et al. 2011). The presence of cyanobacteria in the guts of mummichogs from restored marshes does not indicate that the marshes are not providing ecological functions, but that the marshes not providing the same habitat and food resources that are found in more mature marshes.

Detritus was the most abundant diet component overall in my study (Fig. 5). The presence of detritus is not unusual and is well documented in numerous mummichog diet studies (Prinslow et al. 1974, Kneib and Stiven 1978, Kneib 1986, Moy and Levin 1991, Allen et al. 1994, James-Pirri et al. 2001, McMahon et al. 2005, Thompson 2015). Detritus can be more abundant in either restored or natural marshes (Moy and Levin 1991, Allen et al. 1994, James-Pirri et al. 2001). Diatoms appeared frequently as a diet component of fish from restored sites, but they are not particularly indicative of recently

restored marshes (James-Pirri et al. 2001). Benthic microalgae include diatoms and cyanobacteria and many mummichog diet studies do not separate the two. But many studies agree that benthic microalgae are important primary producers in salt marshes (Sullivan and Moncreiff 1990, Currin et al. 1996, Piehler et al. 1998, Galván et al. 2008, Pennings et al. 2012, Langman et al. 2012). Hodges Creek was the only site without a clear benthic microalgae presence in the diets of mummichogs.

Crab and eggs were more frequently found in the guts of fish from reference sites. The presence of crab and egg in fish diet is indicative of a carnivorous lifestyle and are reflective of a natural marsh (Moy and Levin 1991, James-Pirri et al. 2001, Thompson 2015). However, I found eggs in fish from all restored sites and crab in fish from two restored sites. The reference marshes may have had a greater density of crabs and egg laying organisms (most eggs appeared to be gastropod) and the restored marshes may not have had the same fauna density. The diets of mummichogs from restored and reference marshes in the Southern Branch suggests that restored sites had similar prey items available, but either the prey was not as abundant as in reference marshes or the presence of cyanobacteria was impeding the mummichog's ability to capture prey. It is also possible that heavy metal contamination was impacting mummichog feeding (Weis et al. 2003). While PAHs have not been found to reduce mummichog prey capture ability, disease caused by PAHs may keep mummichogs from feeding normally. The benthic community of the restored marshes may be the similar to the reference marshes and the mummichogs is the contaminated areas are unable to capture prey effectively. It is also possible that benthic community of the restored marshes may have limited abundance because of the sandy conditions, but PAHs also can reduce benthic communities.

Chemical analysis of sediments from the restored and reference marshes could help explain the diets of mummichogs within the marsh systems.

Comparison of Muscle and Liver Stable Isotope Values

I collected muscle and liver tissue for stable isotope analysis because muscle and liver have different turnover times, allowing researchers to determine how quickly diet alters the stable isotope composition of an organism (Fry 2006, Logan et al. 2006, Haas et al. 2009). To measure change over time, samples must be collected over a period of time, which I did not do. Analysis of liver and muscle tissue from one time can still be useful because if the organism does not have a varied diet, the liver and muscle tissue should have the same stable isotope values (Haas et al. 2009). A comparison of $\delta^{13}C$ and $\delta^{15}N$ values from muscle and liver tissue from my study sites shows that liver tissues range from 1‰ to 1.8‰ more depleted in $\delta^{15}N$ than muscle tissue. The most depleted site was Hodges Creek; the site depleted by 1‰ was Money Point. There was not a large difference between restored sites and reference sites. There was not a large shift in $\delta^{15}N$ values in recent diet and less recent diet. Liver tissues ranged from 1.7‰ (Scuffletown) to 4.2‰ (Milldam Creek) more depleted in $\delta^{13}C$ than muscle tissues. The other two restored sites and reference sites were more depleted by ~2.5‰. Primary producers can have $\delta^{13}C$ signals with wide ranges, so the greater depletion in $\delta^{13}C$ values from the liver tissue does not necessarily mean that the more recent diet had a different primary carbon source than the less recent. More research would be very beneficial to improving the usefulness of dual tissue stable isotope analysis for diet determination. To compare my

results to other studies, I have only used stable isotope values from muscle tissues because there are very few studies using mummichog liver tissue at this time.

Potential Carbon Sources in Restored and Reference Salt Marshes

Milldam Creek and Hodges Creek were significantly more depleted in $\delta^{13}C$ than Newton Creek and the restored sites. The $\delta^{13}C$ values indicate the primary source of carbon within a system. *Spartina alterniflora,* upland C3 plants such as *Phragmites* spp., benthic microalgae, and other producers are all possible sources of carbon in the Southern Branch (Table 5). When I compared the $\delta^{13}C$ values from mummichogs I collected to $\delta^{13}C$ values from the literature, it became apparent that there were at least two different primary sources of carbon for my study sites (Fig. 12). Milldam Creek and Hodges Creek $\delta^{13}C$ values are similar to phytoplankton $\delta^{13}C$ values (Fig. 12). Phytoplankton are an important primary source of carbon based on stable isotope analysis of salt marsh producers (Stribling and Cornwell 1997).

Macrophytes appeared to contribute very little to the diet of mummichogs in the restored and reference sites (Fig. 12). However, stable isotope values from liver tissue from my study indicate that Milldam Creek and Hodges Creek have enriched $\delta^{13}C$ values compared to C3 plant values. It is possible that that Milldam Creek and Hodges Creek have multiple important carbon sources from the phytoplankton and run off from upland plants, but due to isotope mixing, the carbon signal from this system is a blend of two or more sources (Fry 2006). The primary carbon source(s) for Newton Creek, Scuffletown, Atlantic Wood, and Money Point appear to be benthic microalgae (Fig. 12). Benthic microalgae include diatoms, cyanobacteria, and filamentous algae. Benthic microalgae

are an important carbon source in multiple salt marsh studies (Mann 1988, Sullivan and Moncreiff 1990, Piehler et al. 1998, Currin et al. 2003, Pennings et al. 2012).

The $\delta^{13}C$ values reflect the presence of cyanobacteria, so gut content analysis and stable isotope analysis seem to agree that cyanobacteria were an important carbon source. Young newly restored salt marshes have cyanobacteria as a main source of carbon because the sediment has not developed detritus and open, sandy conditions benefit cyanobacteria (Currin et al. 1996, Piehler et al. 1998, Watermann and Hillebrand 1999, Zheng et al. 2004, Pennings et al. 2012). The presence of cyanobacteria indicates that the restored salt marshes were not functionally the same as the reference marshes, but the restored marshes were functioning similarly to other studied young marshes. Fish from Newton Creek did not have any cyanobacteria in their guts, so the $\delta^{13}C$ values from Newton Creek being close to the values from restored sites is not because of cyanobacteria presence.

Newton Creek

The similarity of the Newton Creek site to the $\delta^{13}C$ values of the restored sites suggests that benthic microalgae are the primary source of carbon in the system. While stable isotope analysis is unable to clarify the source, the diet of fish from Newton Creek may point out possibilities. Newton Creek fish consumed detritus (which could reflect almost any producer) and diatoms. Newton Creek, Atlantic Wood, and Scuffletown were also the only sites to have filamentous algae in gut contents, although as a minor component. Diatoms and filamentous algae may be enough to result in $\delta^{13}C$ values in the range of benthic microalgae values. Mummichogs from the Newton Creek site consumed

the most insect larvae, including chironomids, which feed on cyanobacteria (Currin et al. 2011). The primary carbon source of the mummichog prey may be causing the $\delta^{13}C$ values of the fish.

It is unclear why Newton Creek is different from the other two reference sites. All sites are located in heavily industrialized and urban areas. The reference sites were chosen because they are thought to be upstream of the PAH contamination from creosote in the Southern Branch. It is possible that Newton Creek had other sources of contamination that I was unaware of when I chose it as a reference site. PAHs can come from petroleum products, including automotive run off (Kimbrough and Dickhut 2006). The reference sites have similar proximities to roads, but there is an auto shop and a used car lot very close to my sampling site at Newton Creek. If petroleum products from either business are running off into Newton Creek, the site may have PAH contamination. PAH contamination would influence the benthic community that the mummichog preys on, potentially altering stable isotope signals and making the site more similar to the restored sites than the other reference sites.

Trophic Position of Mummichogs in Restored and Reference Salt Marshes

The differences in $\delta^{15}N$ values was less than 3‰, indicating that fish from the restored and reference salt marshes are feeding within the same trophic level. Literature values for mummichog muscle tissue show that $\delta^{15}N$ values can range from ~8‰ to ~13‰, with of average of ~9‰ (Currin et al. 1995, 2003, Hughes et al. 2000, McMahon et al. 2005, Wozniak et al. 2006). The $\delta^{15}N$ values for muscle in my study range from 11.1‰ to 12.6‰, indicating that fish from all sites are feeding within the expected

trophic range of mummichogs. Based on $\delta^{15}N$ values, the restored salt marshes are able to support a benthic community well enough to provide food for mummichogs.

TABLE 4. Summary of published stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for primary producers in salt marsh systems.

Primary producer	**Location**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Source**
Spartina alterniflora				
Spartina alterniflora leaves	New Jersey	-12.45	11.78	(Currin et al. 2003)
Spartina alterniflora leaves	New Jersey	-13.25	11.54	(Currin et al. 2003)
Spartina alterniflora (live)	New England	-13.3	5.7	(Wozniak et al. 2006)
Spartina alterniflora (live)	New England	-13.3	6.3	(Wozniak et al. 2006)
Spartina alterniflora (live)	New England	-13.0	7.7	(Wozniak et al. 2006)
Spartina alterniflora (live)	South Carolina	-14.27	4.3	(Couch 1989)
Tall *Spartina alterniflora*	Massachusetts	-13.7	6.3	(Galván et al. 2011)
Spartina alterniflora	Sapelo Island, Georgia	-12.9	6	(Peterson and Howarth 1987)
Spartina alterniflora	Massachusetts	-13.2	7.4	(Galván et al. 2008)
Spartina alterniflora live	North Carolina	-13	5.3	(Currin et al. 1995)
Group Means		**-13.15**	**7.23**	
SE		**0.05**	**0.25**	
C3 plants				
Phragmites spp. leaves	New Jersey	-25.42	7.57	(Currin et al. 2003)
Phragmites spp. leaves	New Jersey	-25.74	10.51	(Currin et al. 2003)
Juncus gerardii	New England	-27.4	3.5	(Wozniak et al. 2006)
Upland C-3 plants	Sapelo Island, Georgia	-29.3	0.4	(Peterson and Howarth 1987)
Phragmites australis	Monie Creek, Chesapeake Bay	-25.32	-	(Stribling and Cornwell 1997)
Juncus roemarianus	Monie Creek, Chesapeake Bay	-27.34	-	(Stribling and Cornwell 1997)

TABLE 4. Continued

Group Means		**-26.88**	**5.50**	
SE		**0.26**	**1.12**	
Benthic microalgae				
Benthic microalgae	New Jersey	-17.17	5.89	(Currin et al. 2003)
Benthic microalgae	New Jersey	-20.92	8.94	(Currin et al. 2003)
Primary producer	**Location**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Source**
Benthic microalgae	South Carolina	-12.11	3.8	(Couch 1989)
Benthic microalgae	Massachusetts	-19.4	5.7	(Galván et al. 2011)
Microphytobenthos	Massachusetts	-19.2	6.0	(Galván et al. 2008)
Benthic microalgae	North Carolina	-13	-0.1	(Currin et al. 1995)
Benthic microalgae	North Carolina	-17.6	-1.1	(Currin et al. 1995)
Group Means		**-17.06**	**4.06**	
SE		**0.47**	**0.51**	
Diatoms				
Epiphytic diatoms	Massachusetts	-20.9	6.1	(Galván et al. 2011)
Benthic diatoms	California	-16	3.5	(Currin et al. 2011)
Benthic diatoms	California	-17.6	4.7	(Currin et al. 2011)
Benthic diatoms	New England	-18	3.7	(Hughes et al. 2000)
Benthic diatoms	Monie Creek, Chesapeake Bay	-14.85	-	(Stribling and Cornwell 1997)
Group Means		**-17.47**	**4.5**	
SE		**0.46**	**0.30**	
Cyanobacteria				
Cyanobacteria	California	-16.1	-0.63	(Currin et al. 2011)
Cyanobacteria	New England	-21	4.3	(Hughes et al. 2000)
Cyanobacteria	California	-17.7	5	(Kwak and Zedler 1997)
Cyanobacteria	California	-15.3	3	(Kwak and Zedler 1997)
Group Means		**-17.53**	**2.92**	
SE		**0.63**	**0.63**	
Filamentous algae				
Filamentous algae	Massachusetts	-18.2	6	(Galván et al. 2011)
Filamentous algae	New England	-	5.8	(Hughes et al. 2000)
Filamentous algae	Massachusetts	-18.3	5.3	(Galván et al. 2008)
Group Means		**-18.25**	**5.7**	
SE		**0.04**	**0.12**	
Phytoplankton				
Suspended Particulate Matter	New Jersey	-23.1	5.93	(Currin et al. 2003)

TABLE 4. Continued

Suspended Particulate Matter	New Jersey	-23.37	7.98	(Currin et al. 2003)
Suspended Particulate Matter	New England	-19.7	4.4	(Wozniak et al. 2006)
Suspended Particulate Matter	New England	-19	7.5	(Wozniak et al. 2006)
Phytoplankton	Massachusetts	-21	12	(Galván et al. 2011)
Primary producer	**Location**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Source**
Phytoplankton	Monie Creek, Chesapeake Bay	-24.08	-	(Stribling and Cornwell 1997)
Suspended Particulate Organic Matter	Massachusetts	-23.7	8.9	(Galván et al. 2008)
Phytoplankton particulates	North Carolina	-20.3	6	(Currin et al. 1995)
Group Means		**-21.73**	**7.66**	
SE		**0.21**	**0.29**	

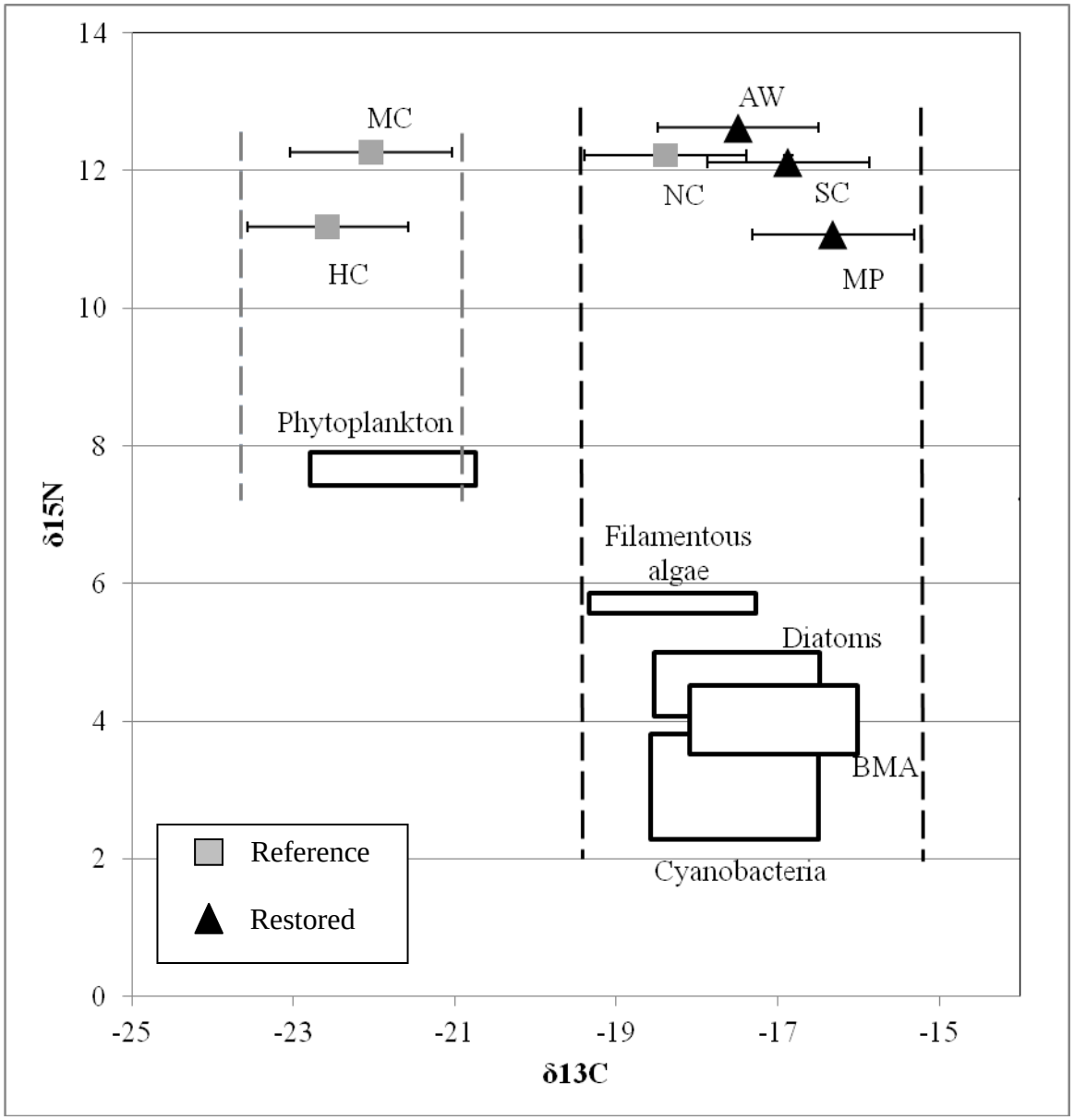

FIG. 12. Average stable isotope values (‰) of nitrogen and carbon from mummichog muscle tissue for each restored site (AW, SC, & MP) and reference site (MC, NC, & HC) from the Elizabeth River with literature stable isotope values from Table 4. Each box represents the edges of SEM bars. BMA stands for benthic microalgae.

CHAPTER 5

CONCLUSIONS

The purpose of my research was to determine if restored salt marshes within the Southern Branch of the Elizabeth River have reached the functional equivalency of natural salt marshes in the same river system. I used *Fundulus heteroclitus* as a tool to monitor the functions of three restored marshes and three reference marshes. Mummichogs from restored sites had higher Gut Fullness Indices, but the mummichogs guts were full of cyanobacteria. The diet of mummichogs from the reference sites was more dependent on detritus, crabs, and eggs. Fish from the restored sites had diets dominated by cyanobacteria. Cyanobacteria alone are not indicative of a failed restoration. The sandy sediment conditions in a restored salt marsh promote the growth of cyanobacteria. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of mummichog muscle and liver tissue revealed that there was a difference in the assimilation of stable isotopes into the fish tissue, suggesting that a dual tissue analysis over a longer time scale may provide valuable information about mummichog diet. Restored sites and Newton Creek were enriched in $\delta^{13}C$ compared to the reference sites. Benthic microalgae appear to be the primary source of carbon for the restored sites and Newton Creek. The restored sites likely rely on cyanobacteria based on the presence of cyanobacteria in the guts of fish from restored sites. Newton Creek fish may derive carbon from diatoms, detritus, or filamentous algae based on the diet of fish from Newton Creek. However, mummichog prey may be feeding on cyanobacteria and the fish would reflect the carbon signal of their prey. Hodges Creek and Milldam Creek seem to have phytoplankton as a primary carbon source. The $\delta^{15}N$ values suggest that the fish from all sites are feeding at the same

trophic level. Restored marshes benthic communities are able to support mummichogs, but functional equivalency has not been reached because the restored marsh sediments are not developed.

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APPENDIX A

TABULAR VALUES FOR AVERAGE GUT FULLNESS INDICES PERCENTAGES WITH STANDARD ERRORS (GFI ± SEM) FOR EACH SITE IN THE SOUTHERN BRANCH OF THE ELIZABETH RIVER. NUMBER OF FISH (*n*) COLLECTED FROM EACH SITE WAS 16.

	Restored			Reference		
	Scuffletown Creek	Atlantic Woods	Money Point	Milldam Creek	Newton Creek	Hodges Creek
GFI ± SEM	3.4 ± 0.6	3.5 ± 0.5	3.2 ± 0.7	1.7 ± 0.3	2.4 ± 0.3	1.3 ± 0.2

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MS in Biology — Expected Graduation May 2016
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Benthic Ecology Laboratory Old Dominion University 2012- 2015
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Used a variety of sampling gears to collect water quality information (YSI), sediment samples, and benthic invertebrate samples (Young gear and box core) from the Chesapeake Bay and tributaries. Conducted laboratory processing of samples (sediment volatiles and characterization). Identified benthic invertebrates from a range of habitat types to species and measured abundance and biomass. Maintained records of data and entered data into Chesapeake Bay Monitoring Program database.

Volunteer work

Wetland Construction 2014
Participated in planting vegetation for a salt marsh in Norfolk, VA.

Lafayette Riverfest 2014
Helped run the Benthic station in the Science Dome. I talked to people of all ages about organisms collected from the Lafayette River.

Publications and Presentations

2016- Master's Thesis: **Naturalization of salt marsh restoration sites in the Elizabeth River, Virginia, assessed by feeding activity and trophic level of mummichogs (*Fundulus heteroclitus*)**

2015- Master's Thesis Defense: **Naturalization of salt marsh restoration sites in the Elizabeth River, Virginia, assessed by feeding activity and trophic level of mummichogs (*Fundulus heteroclitus*)**

2015- Paul W. Kirk Jr. Wetlands Workshop (poster): **Naturalization of salt marsh restoration sites in the Elizabeth River, Virginia, assessed by stable isotope analysis of mummichogs (*Fundulus heteroclitus*)**

2014- Atlantic Estuarine Research Society (oral presentation): **Naturalization of salt marsh restoration sites in the Elizabeth River, Virginia, assessed by feeding activity and trophic level of mummichogs (*Fundulus heteroclitus*)**